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SAFET .COLI

INTRODUCTION

Examining a lot of iGEM projects, we saw that most of them do not properly make a biosafety system. We confirmed this observation by conveying a survey to all iGEM teams and analyzing their answers. We partitioned in 4 classes the 22 answers to the following question and this guided the proposals on which we worked to improve the iGEM biosafety.

Have you considered the effect of the release of your organism in the environment ?

Assessments:	Our solutions:
"Biosafety is not considered or GEO are harmless"	Biosafety guide for future iGEM teams
"Our GEO will not survive outside the lab"	Study about E. coli survival
"We have although about physical containment: alginate beads, lab, bioreactor"	Design of physical containment
"We worked on biological containment (but generally not achieved): kill-switch	Design of a biological containment

ENVIRONMENT CONTAMINATION

Before elaborating any containment systems to prevent dissemination, we thought it would be interesting to investigate *E. coli* survival in different natural environments: Soil, Seawater and Freshwater.

We contaminated samples of our environments by laboratory strains and followed their survival during 9 days. Here are the results we obtained for PhB1320 for the freshwater environment.

FIGURE A: CFU of contaminated Freshwater by *Escherichia coli* PhB1320 Chloramphenicol resistant, at days 0, 1, 3, 7 and 9 on MCK + Cm plates

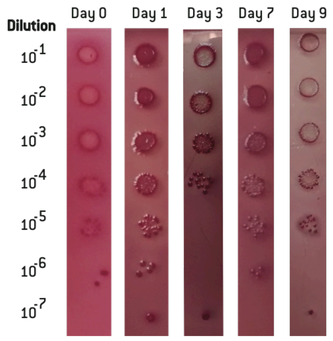
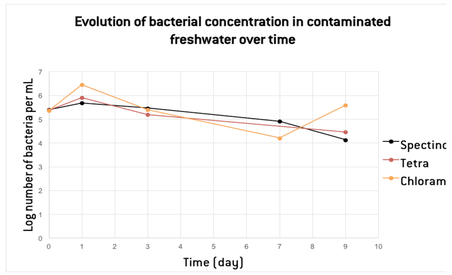


FIGURE B : Log number of bacteria per mL over time in Freshwater contaminated by *Escherichia coli* MG1655Z Chloramphenicol, Spectinomycin or tetracyclin resistant.



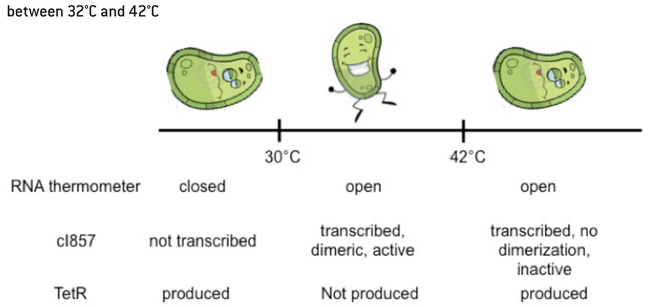
We can conclude that laboratory *E. coli* strains can survive in conditions close to the freshwater environment for at least 10 days. Similar results were obtained for the 3 *E. coli* strains tested and for the other two environment settings tested (seawater and soil).

This experiment shows that laboratory *E. coli* strains can persist in various environments for at least 9 days, and probably longer. Moreover, we did not study here if DNA fragments could be exchanged between organisms, which could be a real issue, especially in case of a prolonged survival in the environment.

BIOLOGICAL CONTAINMENT

Our objective was to control the life or death of *E. coli* bacteria using temperature: we wanted our bacteria to be alive in a defined (working) range of temperatures, and to die when they escape in an environment where the temperature is either higher or lower than the set range of temperatures. In order to create such system, we chose to replace the native promoter of essential genes by a TetR repressible promoter. The production of the TetR protein would then be under a thermal control system constituted of two parts: a RNA thermometer and thermosensitive cI repressor. The thermal system and the tetR/tetRp part would then be integrated in different regions of the chromosome. In the end, when the two mechanisms work together, the bacterial survival is only possible between 32°C and 42°C.

Figure A: when the two mechanisms work together, the bacterial survival is only possible between 32°C and 42°C



PHYSICAL CONTAINMENT

The aim of our physical containment is to let small molecules (nutrients, oxygen) pass through the wall and prevent bacteria from ending up outside. The professor Jacques Livage presented us the work from his team and from another one to help us design a bacterial confinement using silica monolith. As bacteria need space to grow, they are encapsulated first in alginate beads and then put in the solution leading to silica. This material is interesting because it is nano-porous, biomimetic of an algae, the Diatom, which creates glass at room temperature and it can be synthetized by sol-gel process.

Figure B: Obtention of alginate beads with bacterial culture inside

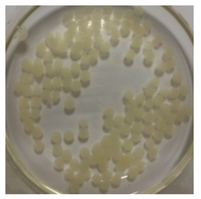
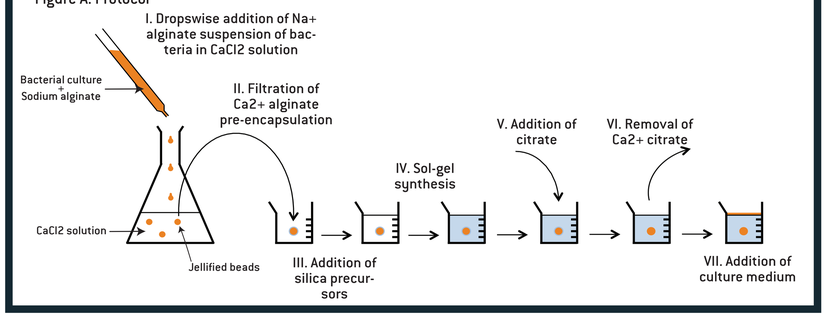
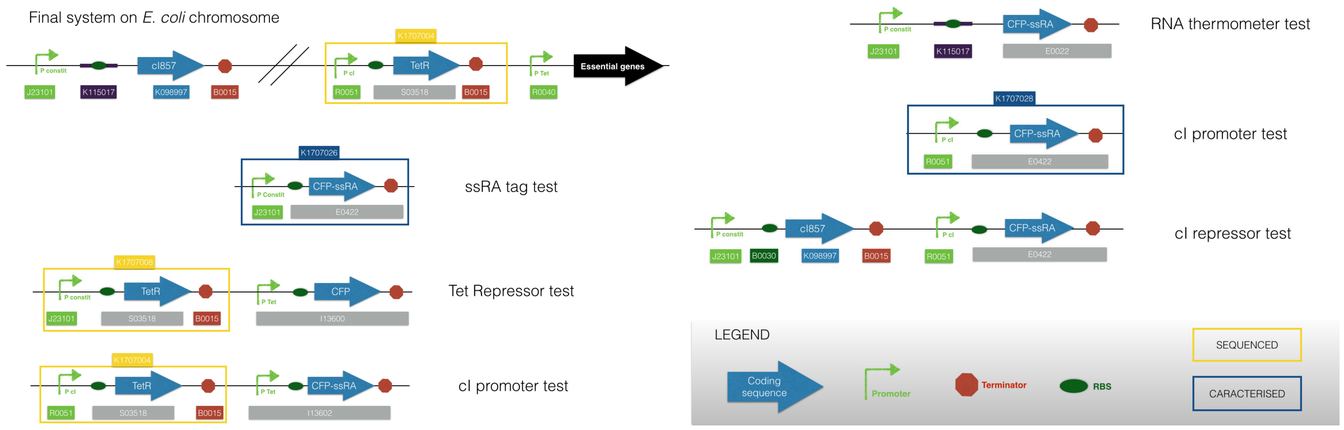


Figure A: Protocol



BIOBRICKS

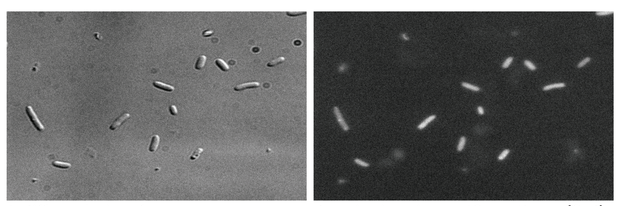
We planned to construct several controls to check that the various parts of our system were properly functioning.



We constructed 37 biobricks. Unfortunately, we encountered some problems and due to a lack of time, we were only able to fully characterize two of them using fluorescence microscopy:

- **BBa_K1707026:** Our control protein CFP-ssRA under a constitutive promoter J23101

Figure A: Characterization of BBa_K1707026 using fluorescence microscopy

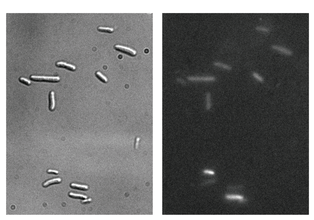


A. Differential interference contrast

B. Excitation with led 437nm/17nm (CFP), 100ms of exposure

- **BBa_K1707028:** Our control protein CFP-ssRA under a repressible promoter cI

Figure B: Characterization of BBa_K1707028 using fluorescence microscopy



A. Differential interference contrast
B. Excitation with led 437nm/17nm (CFP), 100ms of exposure

CONCLUSION AND PERSPECTIVES

We show that laboratory *E. coli* strains can persist in various environments for at least 9 days, and probably longer. To prevent any biological contamination, we conceived a biosafety system based on thermal control. We could imagine tailoring this system for different temperature ranges to meet the user's needs. The cI repressor could be mutated at different positions to try to modify the transition temperature, as could be the switch temperature of the RNA thermometer . In addition, our project is designed to provide the iGEM community with biobricks that could easily be integrated in the chromosome of any *Escherichia coli* chassis with a safety system on. In principle, it could be adapted to other chassis. However, one biological containment system is likely to be insufficient, and to reduce the likelihood of an environmental contamination, it would be better to multiply the biological (auxotrophic selection, toxin/antitoxin system) or physical (such as the silica beads presented here) security systems.

Biosafety Guide for future iGEM teams: